Phase-Shifting Interferometric Analysis of Protein Crystal-Growth Boundaries and Convective Flows

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The objective of the proposed study is to obtain experimental evidence for several characteristics of a crystallizing protein solution and model their effects on the crystal-growth process. The characteristics to be studied include the presence of concentration gradients during the crystal growth, the extent of the boundary layer from the crystal face, and the effect of buoyancy-driven convection on the growth. Phase-shifting interferometry can provide significant insight into this issue, and will produce a visual confirmation of the concentration profile at the boundary layer and concentration values within the depletion region. Phase-shifting interferometry has the potential to offer a direct visualization of convective flows within proteinic crystallizing solutions.

Protein crystals are grown to determine the three-dimensional structure of proteins. By utilizing x-ray or neutron diffraction, the collected information allows the direct identification of macromolecule active sites, their conformation, and the sequence of the amino acids. Sections of very large assemblies of proteins, such as structural proteins or viruses, can also be crystallized. Crystallization is therefore the starting point of any study aimed at the development of new drugs and the understanding of viral diseases. Crystallization techniques for proteins are now well known, but a biophysical understanding of the growth mechanisms is still underdeveloped. This aspect of protein research needs to be expanded, as the proteins being studied are more complex and their purification more costly.

Since the beginning of the 1980's, tens of proteins have been crystallized in microgravity. Microgravity-grown crystals of several proteins were found to be either larger or diffracted with higher resolution than ones previously grown on the ground. These results seem to demonstrate that a reduction of gravity affects the interfacial growth mechanisms that are directly dependent upon the mass-transport regime. Convection is known to play a significant role in the growth kinetics of inorganic crystals, but its importance is still debated in relation to the crystallization of biological macromolecules. There is little doubt about the existence of convective flows in proteinic solutions, but the flow rates they generate close to the crystal/solution interface and their effect on growth kinetics have not been quantified experimentally. The phase-shifting interferometry technique will allow researchers to determine these values and will provide direct comparison between solutal flows in crystallizing solutions under various levels of gravity. An examination of the flows in the fluid and their correlation with crystal growth will strongly depict the role of microgravity in protein crystal growth.

Mach-Zehnder and reflection interferometry, with a magnification of up to 1,000×, will be used to examine growing protein crystals. Phase shifting will be accomplished using an electro-optic phase modulator or a piezo-electric mirror. Abel inverse transforms will be used on the resulting phase maps to provide planar phase information.

Equipment is on order to construct the phase-shifting mechanisms required for the optical systems. Protein crystal-growth material will be provided by MSFC's Biophysics Branch Center.

This study will provide new techniques for viewing protein crystals as they grow. Miniaturization of the optical system could lead to shuttle or space station experiments in microgravity.

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